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Our efforts during the course of this research grant have been devoted to characterizing the role of electrostatic interactions in molecular recognition processes. To this end we have continued to develop and improve our electrostatics methodology which is based on numerical solutions to the Poisson-Boltzmann equation. In addition we have applied this methodology to a number of biochemical processes involved in molecular recognition. These include electrostatically enhanced diffusion, the effect of surface charges on electrical potentials in binding sites, the nature of the electrical potential around nucleic acids, and electrostatic contributions to the folding energies of helical proteins. The latter work has been made possible by a new method we have developed to extract electrostatic contributions to solvation energies from the potentials obtained from the Poisson-Boltzmann equation.

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THE ELECTRICAL POTENTIAL OF PROTEINS

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RESEARCH SUMMARY

The DelPhi program - We have developed our package of programs, known as DelPhi, to the point where it can be distributed to other researchers. It is marketed commercially by BIOSYM and we distribute it ourselves to non-profit institutions. DelPhi calculates the electrical potential of molecules through a numerical solution to the Poisson-Boltzmann equation. The program calculates electrical potentials on a three dimensional lattice as a function of a spatially varying dielectric constant, charge distribution and ionic strength. Recent enhancements in our numerical algorithms make it possible to input the coordinates of a macromolecule and obtain accurate numerical solutions in less than 15 seconds CPU time on a Convex C2 computer.

Electrostaticaly enhanced diffusion - We carried out the first simulation of the diffusion process of a small substrate to the active site of an enzyme. The method we used is known as stochastic or "Brownian" dynamics and involves running a large number of simulated trajectories to determine the probability of a particle hitting its target. The calculations are very time consuming since a large number of trajectories have to be run in order to obtain proper statistics. The method however is quite accurate and can yield results arbitrarily close to those obtained from analytical solutions to the diffusion equation in situations where these are available. Previous studies on enzymes were limited to idealized models of macromolecules such as the use of spheres to represent proteins. Our calculations employed an accurate description of both the protein's shape and its electrical potential. This is made possible through the use of the same grid representation of the protein that is used to solve the electrostatics problem. As a result of our work it is now possible to study the process of protein-substrate recognition using realistic rather than idealized models.

Our initial applications were made to the protein Cu,Zn superoxide dismutase (SOD). We first obtained collision rates for substrate diffusion to the neutral protein and were able to determine

the effect of protein shape on collision rates with the active site Cu. By then introducing the positive (attractive) and negative (repulsive) potentials into the simulations, we were able to evaluate different proposals that have appeared in the literature as to the effect of these factors in guiding substrate diffusion. We found that the repulsive regions of potential have little overall effect while the positive potentials are primarily responsible for enhanced diffusion rates. More importantly, we also succeeded in accounting for the unusually fast diffusion rate of the substrate to SOD as well as for the observed ionic strength dependence of the diffusion process of the native and chemically modified forms of the enzyme. We believe that this work will now make it possible to obtain a far deeper understanding of protein-substrate recognition than has been possible in the past.

Our results have revealed a number of "design principles" of SOD that might be useful in the engineeering of a modified enzyme with enhanced reactivity. Since SOD is widely used as a means of scavenging free radicals in a variety of surgical procedures, our calculations may soon find practical applications. Our most important prediction is that the catalytic rate of SOD can be enhanced dramatically by increasing the positive charge near the opening of the active site channel. Indeed, it appears to be possible to gain a factor of two simply by replacing a single glutamic acid with a lysine. This is clearly a testable suggestion.

pK changes in subtilisin - We published in NATURE that we had succeeded in reproducing the pK changes (including their ionic strength dependence) induced in the active site of subtilisin resulting from site-directed-mutagenesis of residues about 15 Å away. The close agreement between theory and experiment at a range of ionic strengths lends confidence both to our theoretical model and to the precision of the numerical solution to the Poisson-Bolztmann equation. It should be pointed out that in applying the DelPhi program to problems of this type one need not consider the electrical potential of the entire protein but rather the effect of only a single source charge. The protein is treated as a low dielectric region and as such influences the electrical potential of the isolated charge. One of the general conclusions from this study is that electrical interactions tend to proceed though the high dielectric solvent rather than through the low dielectric protein. A useful analogy which makes this behavior understandable is that the protein acts as an insulator while the solvent acts as a conductor.

Charge-solvent interactions with continuum methods - In our previous work we had considered only the electrostatic interactions between pairs of charges. However, in order to calculate the binding energies of charged species or the total conformational energy of a molecule it is necessary to account for the interactions of individual charges with the solvent. For example the loss of solvation energy of charged

substrates upon binding to a macromolecule is approximately energetically equivalent to the gain in Coulombic energy that may drive the association. In a recent paper we showed how it is possible to use the DelPhi program to calculate electrostatic contributions to solvation and binding energies with at least comparable accuracy and orders of magnitude greater computational efficiency than free energy simulations. It was found that charge-solvent interactions, which are frequently neglected in conformational analysis or in calculating binding energies can make extremely large contributions to the total energy of a macromolecular system.

The electrostatic potential of B-DNA - In order to treat nucleic acids as well as proteins, we extended our numerical method so that it could solve the non-linear Poisson-Boltzmann equation. The high charge density of nucleic acids renders invalid the standard assumption that electrical potentials are much less than kT. Electrical potentials around DNA were obtained by solving the non-linear Poisson-Boltzmann equation. The detailed charge distribution and the shape of the dielectric boundary between macromolecule and solvent were explicitly taken into account. Electrical potentials and ion concentrations were compared to those obtained with simpler models. It was found that the shape of the dielectric boundary between the macromolecule and the solvent has significant effects on the calculated potentials, particularly in the grooves. Sequence specific patterns are found, the the most surprising result being the existence of positive regions of potential near the bases in both the major and minor grooves. The effect of solvent and ionic atmosphere screening of phosphate-phosphate repulsions was studied and an effective dielectric constant appropriate for molecular mechanics simulations was derived.

The electrical potential of t-RNA - Calculations of the electrical potentials were made around yeast elongator phenylalanine, aspartate tRNA's and yeast initiator met tRNA in aqueous solution. The initiator tRNA is surrounded by uniformly spaced contours of negative potential. The elongator tRNAs are surrounded by a similar pattern of potentials except in the anticodon region where there is a pronounced 'hole' in the potential surface. The effect of this hole is that the anticodon region is the most positive region of the molecule. The existence of this hole may account for the ability of tRNA to asociate with mRNA.

The stability of α-helical bundles - We calculated the electrostatic work associated with assembling the four helices in the protein hemerythrin to form the observed antiparalle helical bundle. The calculations account for the interaction of each helix dipole with the solvent as well as for pairwise interactions of the dipoles with each other. We found that the electrostatic work of assembly is dominated by unfavorable changes in dipole-solvent interactions rather than by favorable interactions between parallel helices. These results suggest that the helix dipole actually destabilizes the bundle geometry. The

general conclusion that can be derived from this study is that desolvation effects can be as large or even larger than pairwise electrostatic interactions. Thus, in studying molecular recognition it is essential that these effects not be ignored. Conclusions based solely on matching positive and negative regions of potential during a binding process are likely to be grossly incorrect.

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